



INTERACTIVE EFFECT OF 28-HOMOBRASSINOLIDE AND SALINITY ON MORPHO-PHYSIOLOGICAL ATTRIBUTES OF 60 DAY OLD *BRASSICA JUNCEA* PLANTS

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ABSTRACT

Among various environmental stresses, salt stress is an extensively damaging to major crops all over the world. A set of experiments was conducted to examine the role of 28-homobrassinolide on growth and photosynthetic pigments of salt tolerant *Brassica juncea* cultivar *RLC-1* under control or saline conditions i.e., 0 or 180 mM of NaCl. Surface sterilized seeds of *B. juncea* were given pre-sowing soaking treatment of 0, 10⁻⁶, 10⁻⁹, 10⁻¹² M 28-homobrassinolide (28-homoBL) for 8 hours then seeds were sown in pots and were irrigated with 1 liter solution of 180 mM NaCl and control plants were irrigated with distilled water and studied the effect after 60 day after sowing (DAS). Plant growth decreased on imposition of salt stress. Salinity had significant effect on chlorophyll contents and carotenoids content.

Keywords: *Brassica juncea*, Chlorophyll, 28-Homobrassinolide, Salinity, *RLC-1*

I. INTRODUCTION

Biomass productivity is severely affected by a wide range of environmental stresses like drought, alkalinity, salinity and pathogen infections [1]. Soil salinity is one of the major environmental constraints especially in arid and semiarid regions and can limit plant growth and productivity in agricultural systems that rely heavily on irrigation [2, 3, and 4]. Salinity of soil is caused by the occurrence of high amounts of ions in the soil. Presence of high amounts of Na⁺ and Cl⁻ in the soil caused salinity stress. Salinity has many deteriorating effects, viz. it causes disturbances in ion homeostasis, decreases water potential and cause toxicity in plants. This reduction in water balance leads to reduction in plant growth and productivity since, salinity stress involved both ionic and osmotic stresses [5,6]. Growth retardation is directly proportional to osmotic potential and concentration of soluble salts in the soil water [7]. Salinity stress influenced all the vital activities of plants such as photosynthetic efficiency, synthesis of proteins, metabolism of lipids and release of energy. Salt stress results in a stunting growth of plants [8]. Salinity also results in decrease of considerable shoot- root length, fresh and dry weight of leaves [9, 10, and 11].

Plants have developed various strategies for overcoming the salinity stress, these include morphological, physiological and biochemical strategies. Several mechanisms work in a coordinated manner to minimize the

damage due to salinity. Brassinosteroids (BRs) are recognized as a new class of plant polyhydroxy steroid phytohormones, BRs are known for their vital role in plant growth and development. Extensive studies were undertaken worldwide showed ubiquitous presence of BL and their characteristic physiological effects on growth and development of plants as well as their potential abilities in agricultural applications [12]. BRs can protect plants from various biotic and abiotic stresses, such as those caused by salt, high temperatures, and heavy metals [13, 14, and 15]. At cellular level, BRs stimulated elongation, protein and nucleic acid synthesis, enhance photosynthetic capacity and alter mechanical properties of cell wall and permeability of cell membranes. At the whole plant level, they promoted overall growth, reproductive development, shorten the period of vegetative growth, increased crop yield and improved the quality of fruits [16, 17, 18, 19, 20, and 21]. Antistress properties of different active forms of BRs have been suggested by various workers such as salt stress [22], cold stress [23], heat stress [24], and heavy metal stress [25]. The knowledge about this antistress approach will be additional resource information for the dissection of the plant response to salinity, which will further reveal how plants sense salt stress, transduce signals to mediate a defensive response required for stress alleviation, and steady-state growth in the saline environment.

II. MATERIALS AND METHODS

Plant material and growth conditions

Seeds of *B. juncea* L. cultivar (RLC-1) were procured from the Department of Plant Breeding, Punjab Agriculture University, Ludhiana, India. Seeds were surface sterilized with 0.01% HgCl_2 and rinsed 5-6 times with double distilled water. The sterilized seeds were soaked for 8h in different concentrations of 28-homobrassinolide (Sigma-Aldrich. USA) (10^{-6} , 10^{-9} and 10^{-12}M). The treated seeds were propagated in triplicate in cemented pots under natural field conditions. 3 kg soil was added to each pot and 1 L solution of 180 mM NaCl was added to each pot at the time of sowing. Plants were sampled on the 60th day after sowing for measuring various morphological and biochemical parameters.

Growth analysis

Shoot Length (cm)

Shoot length was measured by using a standard centimeter scale on 60th DAS in field during season. The experiment was repeated thrice and the data given here is average value of all the experiments.

Fresh weight and Dry weight (mg)

In field experiments, upper 3rd leaf was taken for fresh weight on 60th DAS. These weighed samples were then dried in oven at 60 ± 2 °C for overnight for dry weight (mg) measurement. For weighing digital balance of AdventurerTM Ohaushing readability 0.001 g was used.

Moisture content (%)

Moisture content (%) was calculated from fresh and dry weight of leaves using following formula:

$$\text{Moisture content} = \frac{\text{Fresh weight} - \text{Dryweight}}{\text{Freshweight}} \times 100$$

Estimation of photosynthetic pigments

Total Chlorophyll, Chl a and Chl b and total carotenoids content in leaves were estimated by the method of Lichtenthaler [26]. 100 mg of plant material was crushed in 80% acetone in dark so as to prevent photo-

bleaching. The homogenate was centrifuged at 3000 x g. The supernatant (I) was collected and residues were re-suspended in 80% acetone and again centrifuge at 3000 x g and supernatant (II) was collected. Both supernatants (I and II) were pooled and optical density was recorded at 645nm, 663 nm and 470 nm by spectrophotometer (UV Mini 1240, Shimadzu) against 5 ml of chilled 80% acetone used as a blank.

Statistical Analysis

All analysis was done on a completely randomized design. All data obtained was subjected to a two-way analysis of variance (ANOVA) and the mean differences were compared by Tukey's test using prism software 5.5. Each data was the mean of three replicates (n=3) and comparisons of p-values <0.05 were considered significant and different from control.

III. RESULTS

Under natural field conditions pre sowing soaking treatment of 28-homoBL ameliorated shoot length (SL) to significant levels over control as well as stressed seedlings and best performance was shown by plants raised from 10^{-9} M treated seedlings, where 84 % enhancement in SL was recorded at 60 DAS. Under field conditions, the data for fresh weight (FW) is presented in (Fig. 1). It has been observed that presowing soaking treatment of 28-homoBL helped in increasing FW of plants (35.42 ± 0.16) at reproductive phase (60 DAS) and this increase in FW was very slow in salt treated (19.21 ± 0.28) plants as well as untreated control plants (25.37 ± 0.08). 28-homoBL treatment enhanced FW of plants to significant levels at reproductive phase. The plants showed higher level of FW in 28-homoBL treated plants, which was significant over control CN DDW and 180 mM NaCl irrigated plants (Fig. 1). Total dry weight (TDW) under field conditions also showed significant enhancement in 28-homoBL treated plants as compared to untreated controlled plants as presented in (Fig. 1). The plants treated with 10^{-9} M 28-homoBL showed regular increase in TDW at 60th days as compared to untreated plants. Other concentrations of 28-homoBL also revealed enhancement in TDW but 10^{-9} M concentration was the best and maximum TDW was recorded (17.36 ± 0.13) at 60th day. Data for moisture content (MC) is presented in (Fig. 1). It has been observed that presowing soaking treatment of 28-homoBL helped in increasing MC of plants, which was very slow in salt treated as well as untreated control plants. 28-homoBL treatment enhanced MC of plants to significant levels at 60 DAS. The plants showed higher MC in 28-homoBL treated plants and statistically significant as compared to control DW plants. Maximum Chl a was recorded in 10^{-9} M concentration of 28-homoBL ($16.05 \pm 0.91 \mu\text{g g}^{-1}$ FW) on 60th DAS as compared to CN DDW plants ($10.52 \pm 0.04 \mu\text{g g}^{-1}$ FW). This amelioration was 52 % more than CN plants. Salt treatment deteriorated the Chl a to significant low levels at 60 DAS in present study. Supplementation of 28-homoBL to plants exposed to salt stress showed ameliorative effect for Chl a and best combination was 10^{-9} M 28-homoBL +180 mM NaCl (13.60 ± 0.40) where 29 % enhancement in Chl a was recorded over CN DDW plants and 17 % more over only salt treated plants on 60th DAS. Maximum Chl b was found ($9.68 \pm 0.27 \mu\text{g g}^{-1}$ FW) on 60 DAS in 10^{-9} M 28-homoBL treated plants, whereas CN plants have only $6.51 \pm 0.21 \mu\text{g g}^{-1}$ FW Chl b, this amelioration was 48 %. Salt treatment deteriorated Chl b to significant low level and impact was sustained throughout the life of plant.

Effect of 28-homoBL form of BRs used in present investigation on total chlorophyll content under field condition at 60 DAS in *B. juncea* plants grown with or without salt treatment is presented in (Fig. 2). Results suggested that total chlorophyll content increased in plants with advent of age. Maximum total chlorophyll

content was found at 60th DAS plants treated with 10⁻⁹ M homoBL (12.73 ± 0.09 µg g⁻¹ FW) as compared to CN plants (4.80 ± 0.12 µg g⁻¹ FW). This enhancement in total chlorophyll content in 10⁻⁹ M treated plants was 165 % more than CN at 60 DAS plants. In present investigations great difference was found regarding the potential of 28-homoBL for ameliorating total chlorophyll content in plants with advent of age as well in plants exposed to salt stress or not.

Under field conditions, the beneficiary effects of different concentrations of 28-homoBL on plants at 60 DAS of growth with or without salt are shown in (Fig. 2). 10⁻⁹ M 28-homoBL also found to be best at 60 DAS of growth and development of plants. Maximum carotenoids was found (9.69±0.03 µg g⁻¹ FW) at 60th DAS in 10⁻⁹ M treated plants whereas CN plants have only 4.63±0.09 µg g⁻¹ FW. Which was 109 % more than CN untreated plants. Salt treatment deteriorated total carotenoids to 19 % over CN seedlings at 60th DAS. While the plants which were supplemented with 28-homoBL showed boosted accumulation of total carotenoids. Best combined treatment was 10⁻⁹M 28-homoBL + salt (7.73±0.09), where 66 % additional total carotenoids was recorded over CN DDW seedlings.

IV. DISCUSSION

Exogenous application of different concentrations of 28-homoBL to plants before exposing them to harsh conditions of seed germination, in present study found to be very supportive for seed germination under laboratory and in soil under field which was upto 36% and 25% respectively. This reassuring demeanor of 28-homoBL for seed germination illustrated the ameliorative property of this BR for seed germination which may be correlated with homeostasis maintenance potential of 28-homoBL. Number of supportive data for our results has been given by [27, 28, 29, and 30] on *Lepidum sativus*, *Eucalyptus camaldulensis*, *Arachis hypogea*, *Brassica juncea*, *Oryza sativa*, *Triticum aestivum*, *Lycopersicon esculentum* and *Orabanchae minor*. Our results confirmed the dose dependent behavior of 28-homoBL for seed germination boosting under different conditions which was synergistic with findings of [31,32,33] who also supported that 28-homoBL at 10⁻⁹ M concentration was the best for germination in *C. arietinum*. These results were attributed to the fact that 28-homoBL enhances α-amylase activity, the main enzyme responsible for mobilization of reserve food during germination process along with triggering the activity of CAT, POX, total soluble sugars (TSS) and protein content of seeds. In this manner, 28-homoBL may mimic with GA signaling pathway for seed germination as reported by [34] in *Arabidopsis*. 28-homoBL is very much synergistic in its behavior with other growth involving signaling pathways during stress to promote growth of plant. Our findings supported the finding of [35] who reported that salt stress declined mitotic index in barley plants consequence to deterioration in plant growth. Research findings of [36] was stand in support of our findings for exposing the anti stress potential of 28-homoBL on root nodulation property of water stressed french bean plants. While, [37] reported the ameliorative property of 28-homoBL on *in vitro* banana shoot cultures over CN untreated cultures. 28-homoBL pronounced the shoot growth of *B. juncea* RLM-619 plants at concentrations of 0.10-0.05mgL⁻¹ over DDW plants with or without temperature stress [38]. 28-homoBL effect for chlorophyll enhancement was increased with advent of growth and it was maximum at 60 DAS although 28-homoBL treatment was given to seeds before sowing. Hence, it can be suggested that 28-homoBL being signaling molecule require sufficient time to trigger the internal immune response machinery and other components of metabolic machinery well in advance and this can occur

when the application of 28-homoBL given to seeds rather than at seedling stage on as spray treatment of 60 DAS plants. Hence, prior treatment of 28-homoBL to seedlings proved to be beneficiary and protect the total chlorophyll as well as chlorophyll *a* and *b* from thermal and ionic oxidation. Our results regarding positive effects of 28-homoBL on total chl are supported by [39, 40] on *Brassica* leaves, who recorded enhancement in total chl by exogenous application of BR⁵. similar results were narrated in mungbean [41, 42, 43]; Indian mustard [44], chickpea [45]. Present results are also supported by [46, 47]. Honnerova [48] who reported enhancing effect of BR on elevation of chl amount in *Phaseolus vulgaris*, cucumber and *Zea mays*. Our results are in close proximity with Bajguz [49] who suggested improvement of total carotenoids in *Chlorella vulgaris* by exogenous application of 28-homoBL but in dose dependent manner. Total carotenoids decreased under salt treatments, this decrease in level of carotenoids such as α -carotene, β -carotene and xanthin might abide the excitation energy of chlorophyll to transmit from photosynthetic reaction to outside which is one of the mechanism adopted by plants to protect chlorophyll from photo-oxidation that lead to degradation in chlorophyll [50].

V. CONCLUSIONS

The influence of 28-homoBL on plant growth and photosynthetic pigments was more prominent under NaCl stress, suggesting that 28-homoBL treated plants was less affected by NaCl than the untreated plants. Also, 28-homoBL induced elevated levels of photosynthetic pigments, which increased the tolerance of *Brassica juncea* plants to NaCl stress. However, the molecular mechanism involved in function of stress protection remains to be explored.

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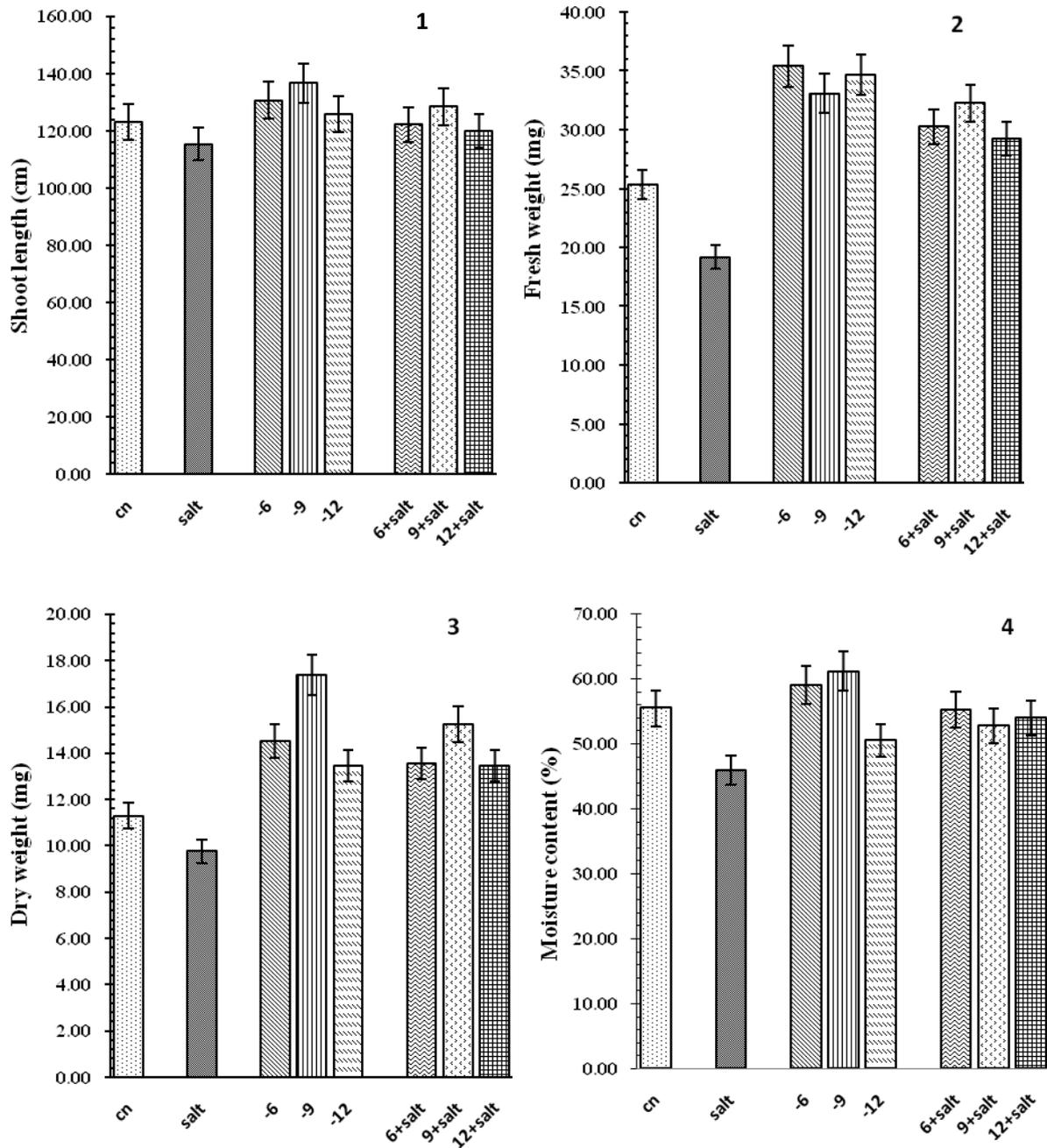


Fig: 1. Effect of different concentrations of 28-homoBL (0, 10⁻⁶, 10⁻⁹, 10⁻¹² M) on (1) shoot length (cm), (2) Fresh weight (mg), Dry weight (cm), Moisture content (%) of *B. juncea* L. under NaCl salt (180 mM) treatment in 60 day old plants under natural field conditions

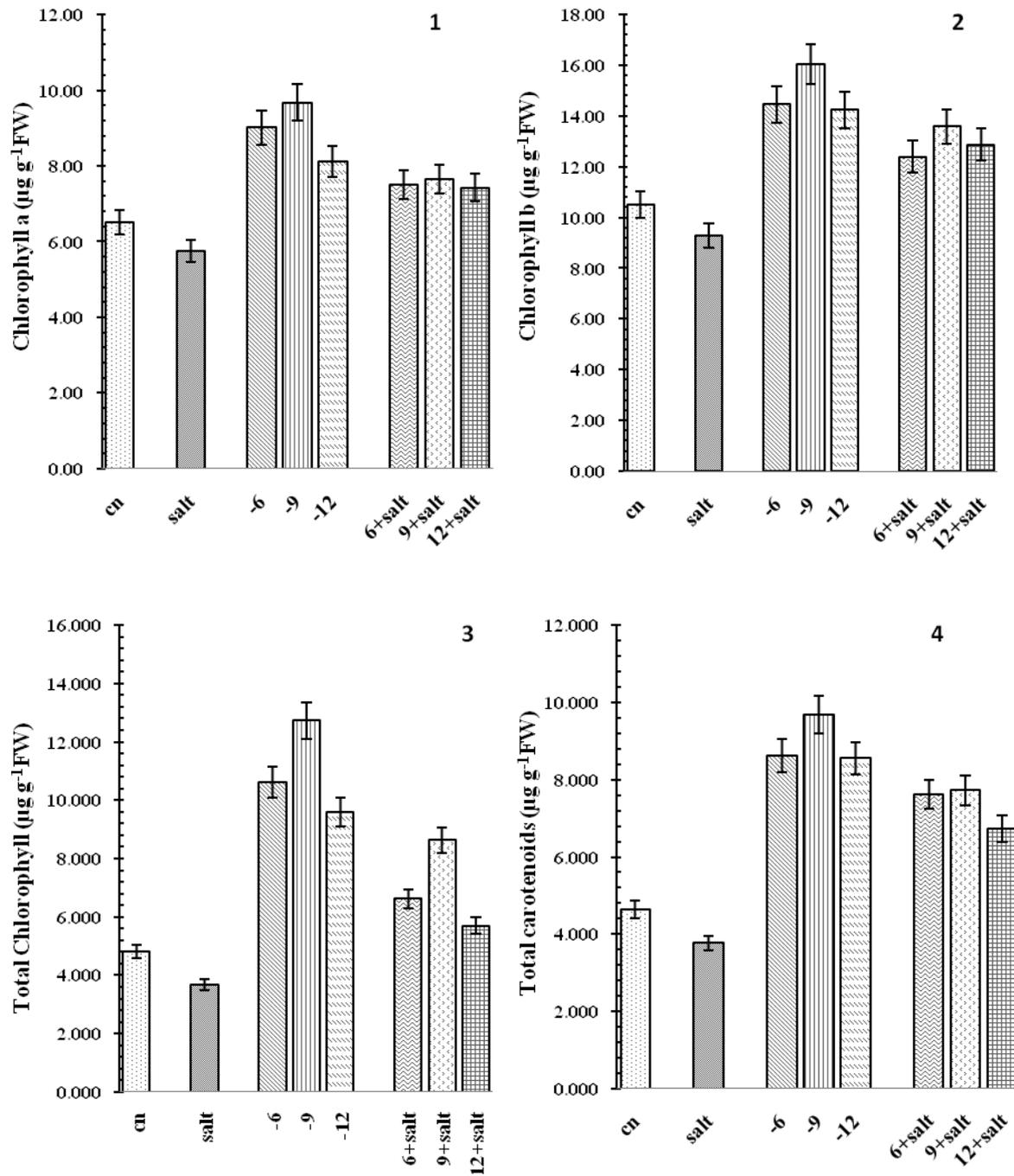


Fig: 2. Effect of different concentrations of 28-homoBL (0, 10⁻⁶, 10⁻⁹, 10⁻¹² M) on (1) chlorophyll a (2) chlorophyll b (3) total chlorophyll (4) total carotenoids content ($\mu\text{g g}^{-1}\text{FW}$) of *B. juncea* L. under NaCl salt (180 mM) treatment in 60 day old plants under natural field conditions